# Seabrook Stormwater Verification Project
# Quality Assurance Project Plan

July 18, 2003

*Prepared by*
Scott S. Nolan
UNH Jackson Estuarine Lab
85 Adams Point Road
Durham NH 03825

---

Project Manager: _____
Signature / Date
Scott Nolan, UNH, JEL

Project QA Officer: _____
Signature / Date
Stephen Jones, Ph.D., UNH, JEL

DES Project Manager _____
Signature / Date
Natalie Landry, NHDES

Program Quality Assurance Coordinator: _____
Signature / Date
Andrea Donlon, NHDES

NHDES Quality Assurance Manager: _____
Signature / Date
Vincent Perelli, NHDES

USEPA Project Manager: _____
Signature / Date
Warren Howard, US EPA Region I

USEPA QA Manager: _____
Signature / Date
Alan Peterson, US EPA Region I

## A2 – Table of Contents



### List of Tables

## List of Figures



## A3 – Distribution List

Table 1 presents a list of people who will receive the approved QAPP, the QAPP revisions, and any amendments.

**Table 1 QAPP Distribution List**

| Name | Project Role | Organization | Telephone number and Email address |
|---|---|---|---|
| Steve Jones | Project QA Officer | UNH Jackson Estuarine Lab | 603-862-2175 shj@cisunix.unh.edu |
| Scott Nolan | Project Manager, Field and Lab technician | UNH Jackson Estuarine Lab | 603-312-0339 snolan@metrocast.net |
| Natalie Landry | DES Project Manager | NHDES Watershed Management Bureau | 603-433-0877 nlandry@des.state.nh.us |
| Vincent Perelli | NHDES Quality Assurance Manager | NH DES Planning Unit | 603-271-8989 vperelli@des.state.nh.us |
| Andrea Donlon | Program QA Coordinator | NHDES Watershed Management Bureau | 603-271-8862 adonlon@des.state.nh.us |
| Jean Brochi | USEP NHEP Project Manager | USEPA New England | 617-918-1536 brochi.jean@epa.gov |
| Warren Howard | USEPA 319 Project Manager | USEPA New England | 617-918-1587 Howard.Warren@epa.gov |
| Alan Peterson | USEPA Quality Assurance Officer | USEPA New England | 617-918-8322 peterson.alan@epamail.epa.gov |

## A4 – Project/Task Organization

This study will be completed by staff from UNH Jackson Estuarine Laboratory. Samples are delivered for laboratory analysis to the UNH Jackson Estuarine Laboratory in Durham, New Hampshire. Steve Jones oversees all facets of laboratory analysis and quality assurance. Scott Nolan will collect and analyze all samples for bacterial indicators, conductivity and pH. He will also be responsible for the overall completion of the project, preparation of the final report, preparation and maintenance of the approved QA Project Plan, and will be the primary contact between UNH, NHDES, and the EPA.

The data generated by this study will be used by NHDES Watershed Assistance Section to evaluate the effectiveness of the AbTech SmartSponge at reducing bacterial concentrations into Hampton Harbor. These data will be made available to the public upon request.

**Figure 1 Project organization chart**

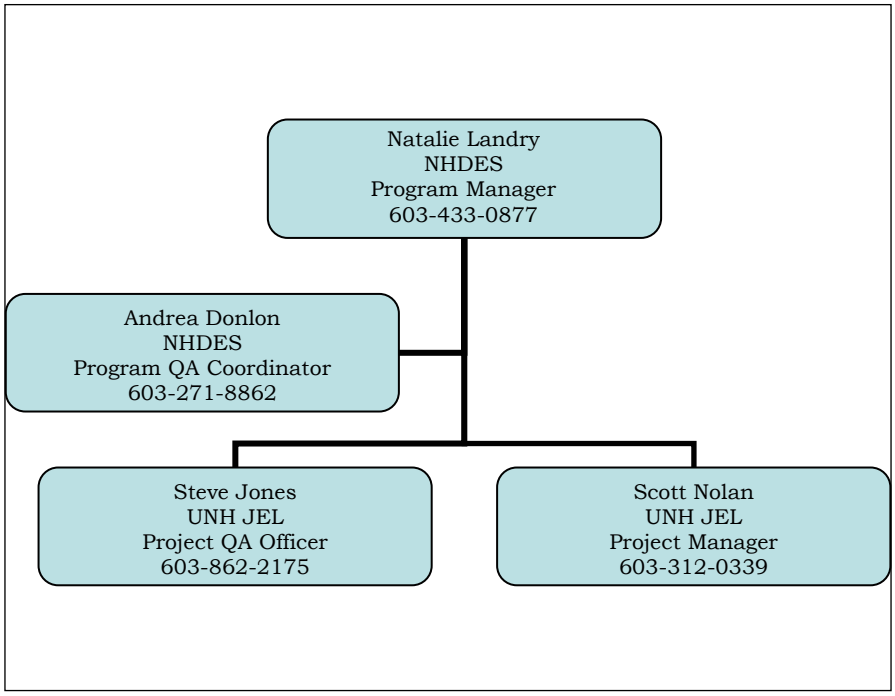



Figure 1 shows an organizational chart for this project. NHDES, via Natalie Landry, will be the principal data users. Dr. Steve Jones is the project QA Manager. Scott Nolan will be responsible for sampling and analyses and will be responsible for tracking changes in and maintaining the QAPP. Dr. Jones will supervise lab and field QA procedures, keep QA records and ensure that the QAPP is updated.

Table 2 below describes primary contacts, coordination of activities and the responsible personnel.

**Table 2 Personnel Responsibilities and Qualifications**

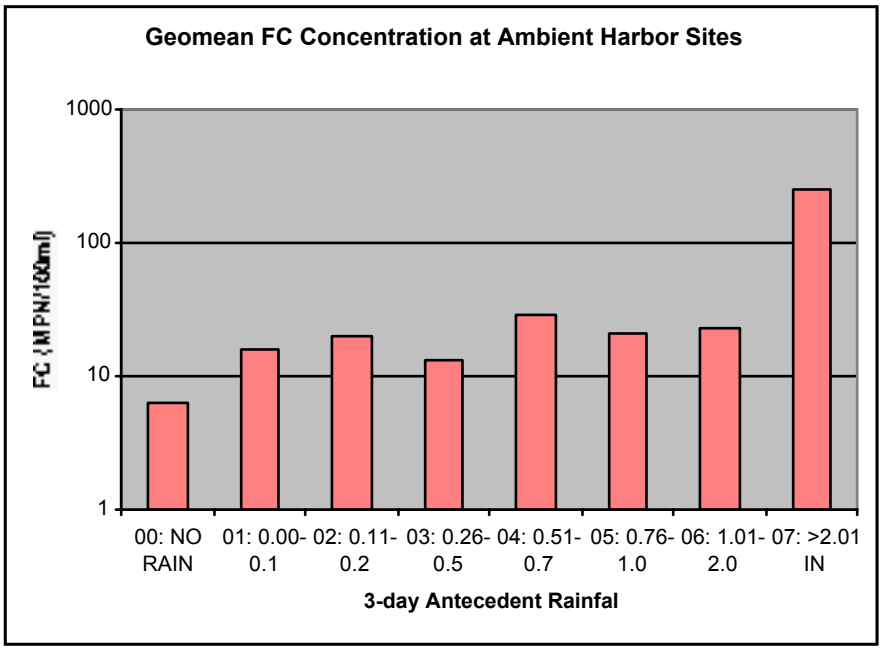
| Name and Affiliation | Responsibilities | Qualifications |
|---|---|---|
| Natalie Landry<br>NH DES Watershed Management Bureau | DES Project Manager: responsible for grant/contract management of the project, providing DES data to the Project Manager, contacting and working with the Town of Seabrook D.P.W. | On file at NHDES |
| Steve Jones | Project QA Officer: responsible for technical assistance to Project Manager and technician, QA oversight, and QA reporting. | Research Professor of Marine Science/Natural Resources, Ph.D. in Bacteriology, 23 years experience in environmental research |
| Scott Nolan | Project Manager: responsible for day-to-day project activities, conducting and overseeing the analysis of data and information, and reporting to DES. | B.S. in Biology, 19 years environmental research experience. |
| Andrea Donlon<br>NH DES Watershed Management Bureau | Reviews QAPP preparation and other QA/QC activities | On file at NHDES |
| Alan Peterson<br>EPA Region I Laboratory | Responsible for review and approval of QAPP | On file at EPA |

Based on EPA-NE Worksheet #6.

## A5 – Problem Definition/Background

Hampton Harbor and its tributaries were included on the New Hampshire Department of Environmental Services (NHDES) 1998 303(d) list of impaired waterbodies due to bacterial pollution (primarily during wet weather) that impairs its use for shellfishing (see Figure 2) (DES, 1998). Bacterial contamination during wet weather events, especially during September-October, has been an ongoing factor limiting the harvest of soft-shell clams in the harbor (Nash, 2002).

**Figure 2 Geometric mean concentrations of fecal coliforms (FC) at Hampton Harbor sites (1988-2001).**




Note: The NSSP standard for geomean FC is 14 MPN/100ml.

Over the past several years, NHDES and other agencies have focused on identifying pollution sources that contribute to wet weather contamination of Hampton Harbor. Jones and Landry (2003) reported a variety of different source species in the harbor based on *Escherichia coli* ribotyping. The most significant source species was humans, suggesting leaks from sewage infrastructure, septic systems, discharges from WWTFs and boats as causes of this pollution source. In October, 2002, sampling of stormwater discharge from the same stormwater pipe targeted for this study was conducted during a 12-h storm in which 1.39" of rain fell (Trowbridge, 2003). *Escherichia coli* concentrations ranged from 14,400 to 1,120,000 cfu/100 ml during the storm, following a pattern of gradual rise to a peak followed by a sharp decline (Jones, 2003). Ribotyping of *Escherichia coli* isolates suggested humans and cormorants were the most significant sources of the bacterial contamination. Even though the effluent sampled included input from other branches of the stormwater system besides the portion targeted in the present study, this report showed how significant the contamination is from this pipe and the dynamics of bacterial concentrations during the storm.

The goal of this project is to evaluate the pollutant removal efficiencies of an AbTech SmartSponge installed in an existing stormwater treatment system using an independent Environmental Technology Verification (ETV) program and to disseminate the findings of the study to New Hampshire stormwater managers. The AbTech material was selected based on its potential ability to reduce bacterial inputs in stormwater. The removal efficiencies will be calculated by collecting site rainfall data, AbTech influent and effluent water samples and stormwater flow data during 15 rainfall events. Refer to the AbTech company information in Appendix B.

This project, using the Draft Verification Protocol for Stormwater Source Area Treatment Technologies Draft 4.1 (EPA, 2002. http://www.epa.gov/etv/pdfs/vp/04_vp_stormwater.pdf ), will test the ability of the AbTech SmartSponge media to reduce bacterial loads into Hampton Harbor from a storm drainage system in Seabrook, New Hampshire. This storm drainage system is of concern because

of the high bacterial loading it discharges to the harbor during storms (Jones, 2003). The stormwater system is comprised of a series of pipes and catch basins on residential streets east of Route 1A and north of Hooksett Street that collect stormwater via leaching basins and perforated and solid pipes to a pump station located on Route 1A across from Tilton Street.(See Figure 3) Stormwater is then pumped via a 16-inch force main to a drain manhole at the intersection of River Street and Route 1A. The AbTech material is placed in an existing water quality inlet situated upstream of the pump station. (See Figure 4).

**Figure 3. Site location map**

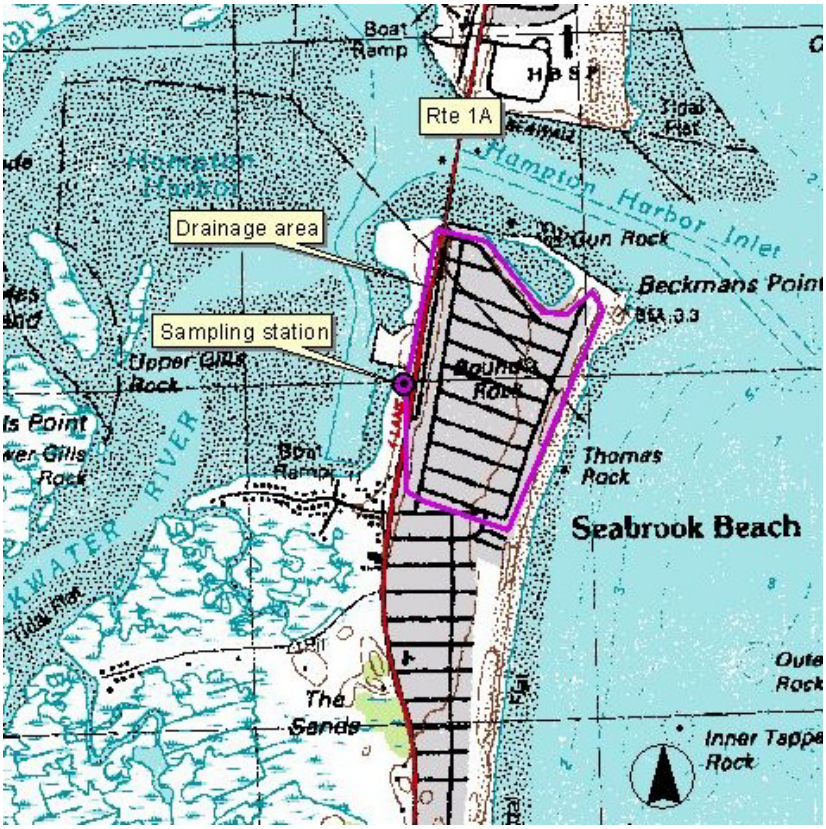
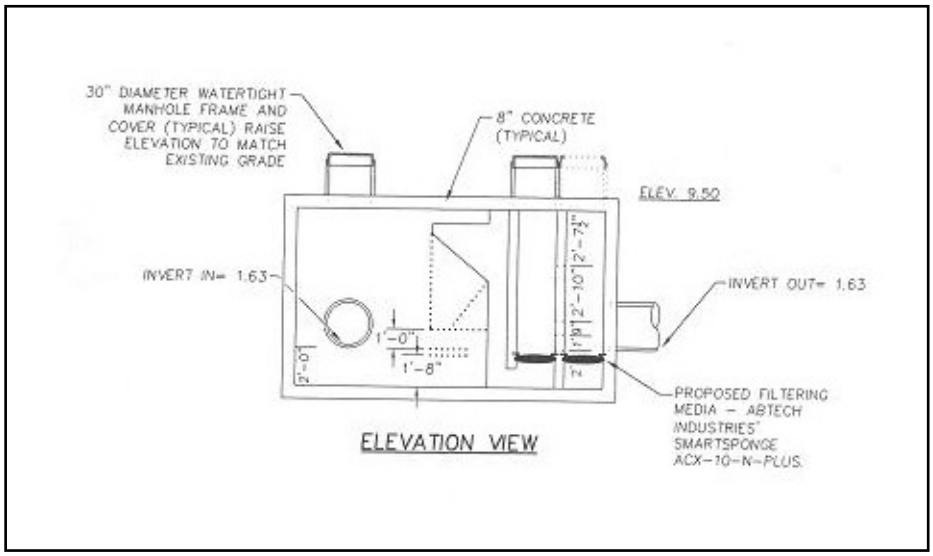



**Figure 4. Side view of existing water quality inlet**



This project is funded through the New Hampshire Estuaries Project and from a DES watershed restoration grant using Clean Water Act Section 319 Money.

## A6 – Project/Task Description

Urban stormwater sampling

An AbTech SmartSponge device installed in an existing water quality inlet at Route 1A in Seabrook will be evaluated for pollutant removal efficiencies based on water quality and quantity data collected during 15 rain events during the summer and fall of 2003. Pollutants to be measured include three bacterial indicators (*E. coli*, Enterococci, fecal coliform). Conductivity and pH will also be measured.

Rainfall intensities and flow data will be measured for several storms before any sampling occurs. All data collected from the testing phase of the project will be kept in a project notebook. An ISCO 674 Rain Gauge will collect rainfall data. Two ISCO 6712FR Refrigerated Samplers will collect the influent and effluent samples and an ISCO 4220 Flow Meter Pressure Transducer will measure flow. When activated the influent and effluent samplers will, on a slightly staggered basis, collect at least five samples each, over the duration of the storm. The effluent sample collection will initiate 5 minutes following the initial influent sample collection. The influent and effluent samples will be tested for bacterial indicators, conductivity and pH. Flow and rainfall data will be collected simultaneously with the water samples, which will provide the data necessary to calculate pollutant removal efficiencies.

Data analysis, interpretation and reporting

The data from sample analyses for bacterial indicators, conductivity and pH will be compiled into electronic spreadsheets for analysis and interpretation. Following review, verification and validation, the data will be analyzed to determine two primary performance indicators to characterize the pollutant removal efficiency of the stormwater treatment technology based on pre- and post-treatment sample data: (1) an efficiency ratio based on reduction in event mean concentration of the pollutant in the flow for each storm event, and (2) a load reduction based on the percentage of the total amount of pollutant removed (see section C2 for more details). Both indicators shall be calculated and reported for the individual storms monitored and as averages over all the sampled rainfall events. These indicators shall be developed for each pollutant constituent monitored.

Quarterly reports (9/31/03, 12/31/03, and 3/31/04) to NHDES will include descriptions of all work and all collected data. The final report (6/30/04) will be based on the data analyses and interpretations done as described in this QAPP and the project proposal. The final report will include introductory and summary information, and all pertinent illustrations and graphical representations of the data. A report on the success of the quality assurance and control will also be included in the final report. The expiration date for the contract is 6/30/04.

**Table 3. Project Schedule Timeline**

| Activity | Dates (MM/DD/YYYY) | | Product | Due Date |
|---|---|---|---|---|
| | Anticipated Date(s) of Initiation | Anticipated Date(s) of Completion | | |
| QAPP Preparation | August, 2002 | July 20, 2003 | QAPP Document | July 20, 2003 |
| Installation of monitoring equipment | October, 2002 | October, 2002 | All monitoring equipment installed at site | October, 2002 |

| Activity | Dates (MM/DD/YYYY) | | Product | Due Date |
|---|---|---|---|---|
| | Anticipated Date(s) of Initiation | Anticipated Date(s) of Completion | | |
| Installation of Ab-Tech media | April, 2003 | April 2003 | Install Ab-Tech media in –line at site | April 2003 |
| System testing | May, 2003 | July 2003 | Testing of monitoring and media methods | July, 2003 |
| Sample collection | July, 2003 | December, 2003 | Samples for analysis of pH, conductivity, and bacteria. | December, 2003 |
| Sample analysis | July, 2003 | January, 2004 | Results for pH, conductivity, and bacteria. | January, 2004 |
| Data analysis & interpretation | July, 2003 | February, 2004 | Organized database and interpretations | February, 2003 |
| Quarterly project report preparation | September 1, 2003 | September 30, 2003 | Quarterly project report | September 31, 2003 |
| Quarterly project report preparation | December 1, 2003 | December 31, 2003 | Quarterly project report | December 31, 2003 |
| Quarterly project report preparation | March 1, 2004 | March 31, 2004 | Quarterly project report | March 31, 2004 |
| Final project report preparation | June 1, 2004 | June 30, 2004 | Final project report | June 30, 2004 |

## A7 – Quality Objectives and Criteria

Table 4 summarizes the performance criteria for field collection and analysis of bacterial indicators in samples collected for this project.

**Table 4 Measurement Performance Criteria for Bacterial Indicators.**

| Data Quality Indicators | Measurement Performance Criteria | QC Sample and/or Activity Used to Assess Measurement Performance |
|---|---|---|
| Precision-Field | RPD≤ 20% | Field Duplicates |
| Precision-Lab | R< precision criterion (see text below) | Lab duplicates |
| Detection limits | 1 cfu/100 ml | Sterility tests |
| Accuracy/Bias | Positive results with positive controls<br>Negative results with negative controls | Positive and negative controls |
| Comparability | Deviation from SOPs should not influence more than 5% of the data | Data Comparability Check |
| Sensitivity | Not expected to be an issue for this project | |
| Data Completeness | 80% samples collected | Data Completeness Check |

Based on EPA-NE QAPP Workbook for 3/19/02 DES QAPP writing class.

**Table 5. Measurement performance criteria**

| Parameter | Meas. Range[2] | Precision | Accuracy | Reporting Limit |
|---|---|---|---|---|
| pH | 4 - 10 units | ± 0.2 units | --- | --- |
| Conductivity | 0-1999µS<br>0-199mS | 1µS, .01mS | ±1% Full scale | 1 µS |
| Bacterial Indicators | >= 1cfu/100mls-10 | 1 cfu/100mls | 1 cfu | 1 cfu |

Precision & Accuracy/Bias: Field precision will yield RPDs ≤ 20%. If RPD routinely exceeds 20%, acceptable level may need to be adjusted. This will be noted in the final report. Relative percent difference (RPD) will be calculated:

$$RPD = \frac{X_1 - X_2}{\frac{X_1 + X_2}{2}} \times 100$$

Laboratory precision for bacterial indicator measurements is typically determined according to Standard Methods 9020 B-8. (APHA, 1998). The range (R) for duplicate samples is calculated and compared to predetermined precision criteria. The precision criterion is calculated from the range of log-transformed results for 15 duplicates according to the following formula:

3.27 × (mean of log ranges for 15 duplicates) = precision criterion

The precision criterion is updated periodically using the first 15 duplicate samples analyzed in a month by the same analyst. If the range of ensuing pairs of duplicate samples is greater than the precision criterion, then the increase in imprecision will be evaluated to determine if it is acceptable. If not, analytical results obtained since the previous precision check will be evaluated and potentially discarded. The cause of the imprecision will be identified and resolved.

Representativeness: The objective of this study is to make measurements that will be representative of the loading of bacteria from storm drains around Hampton Harbor. To that end:

- The storm drain system selected for the study was chosen because its size is typical of other systems in the watershed; previous sampling data indicated elevated bacteria concentrations; and its close proximity to ambient harbor stations. As a result, this storm drain is expected to be representative of the major stormwater sources of bacteria to the harbor.
- To be representative of the stormwater loading, this study follows the outline of sampling criteria outlined in the US EPA Environmental Technology Verification Program Verification protocol for Stormwater Source Area Treatment Technologies Draft 4.1 Publication (ETV). Autosamplers will be programmed to begin sampling during the first flush and will take flow-weighted samples during the event. The samples will then be sub-sampled into a composite sample using flow weighted measurements from the hydrograph, as required by the ETV.

Comparability: There have not been any studies conducted in New Hampshire that are similar to this one. The laboratory involved in the study has conducted the same types of analyses on samples from stormwater runoff in coastal New Hampshire in the past and will be in the future, using the same equipment and analytical methods. Thus, the analytical results from this study should be comparable to other studies conducted by the lab. The results will also be compatible with other technology treatments using the same ETV.

Completeness: This study proposes to monitor a total of fifteen storms between July 2003 and December 2003. Depending on numbers of events, it may be necessary to increase or decrease the time needed to capture 15 storms.

Sensitivity: There is no defined 'level of interest' that we are seeking for concentrations of indicator organisms. The data will define if the material removes the indicator organisms. For indicator organisms, we are interested in whatever range we can detect. However, at the low end, concentrations that are below the detection limit of 1 cfu/100 ml are not of interest because of the low level of loading to estuarine waters that such numbers represent. To increase the sensitivity of bacterial analysis, filtration of larger volumes would be required. However, volumes >100 ml typically cause clogging problems with membrane filters and will be avoided if at all possible.

Quantitation Limits: The analytical method, analytical/achievable method detection limit, and the analytical/achievable laboratory quantitation limits for this project are shown in Table 8.

## A8 – Special Training/Certification

The project tasks will be performed by experienced staff comprised of UNH personnel. Table 2 lists the qualifications of the project staff.

## A9 – Documents and Records

The Project Manager will be responsible for maintaining the approved QA Project Plan and for distributing the latest version of the plan to all parties on the distribution list in Table 1 (Section A3). A copy of the approved plan will be on file at the DES Watershed Assistance Section office in Concord, and at the JEL Microbiology lab.

A Collection Data Log Sheet will be completed by the project manager for each set of samples collected during a rain event. Copies of all Collection Data Log Sheets are kept by the Project Manager. A copy of the log sheet to be used is included in Appendix D.

Quarterly reports will be prepared by UNH for DES, which will include two quarterly reports outlining the accomplished activities during the past three months and any data that have been collected. Upon completion of the project, a final report will be prepared and submitted to DES. The final report will include all data and analyses performed and will provide conclusions and recommendations as necessary. The quarterly and final reports will be in electronic and hardcopy format.

All data and records will be stored in the offices of the project manager and the QA officer. Data and records will remain accessible to DES for at least three years following the completion of the project.

## B1 – Sampling Process Design

Samples will be collected using ISCO Model 6712 refrigerated samplers with 24 discrete bottles at two locations within the stormwater discharge system. Refer to "Use of Automatic Samplers for Bacterial Sampling" in Appendix C for automated sampler use justification. One sampler will collect samples from a pre-treatment location upstream of the SmartSponge treatment material. The other sample will be collected from a site downstream of the SmartSponge.

Criteria for Qualified Sampling Event

For an event to be considered a qualified sampling event, the following conditions shall be met, according to US EPA Environmental Technology Verification (ETV) Program.

1. The total rainfall depth for the event, measured at the site, shall be 0.2 inches or greater;
2. Flow through the treatment device shall be successfully measured and recorded over the duration of the runoff period.
3. There shall be a minimum of 6 hours between qualified sampling events. For the purposes of this study, there is no minimum required antecedent dry weather period.
4. Samples will be taken during any and all events and considered a qualified sample if it meets the above criteria.

Composite Sampling

Samples will be collected based on flow and time. Each autosampler will be programmed to collect a minimum of five samples over the course of the storm. Each discrete sample will be collected in a 1000ml bottle and refrigerated. Within 6 hours from the first discrete sample to be used in the composite samples, all of the samples will be transported to the lab and the final composite sample of 5 discreet samples will be generated using the hydrograph. A flow-weighted calculation will be used to determine the proper aliquots needed from each discrete sample and a single composite sample, from each sampler, will be analyzed for *E. coli*, fecal coliform, enterococci, pH and conductivity. The bacterial indicators are critical measurements; the pH and conductivity are needed only for information.

## B2 – Sampling Methods

Samples will be collected using ISCO Model 6712 refrigerated samplers. Flow measurements will trigger both of the 6712's and samples will be collected on the rising limb, peak flow, and the declining limb of the event. The samples will be collected in sterile 1 liter PE bottles and kept at 4° C until transported to JEL. Holding times for all analyses are listed in Table 6.

Preliminary Rain and Flow Measurements

An Isco Model 674 rain gauge has been installed at the site, secured to a pole at least six feet above ground level, in an open area away from trees and structures impacting rainfall measurements. (See Figure 5) The rain gauge is powered by the flow meter. A cable connects the rain gauge to the flow meter. The rain gauge transmits a signal via the cable for each tip of the collector which represents 0.01 inches of rain. Prior to any samples being collected, the rain gauge and flow meter will be monitored during three storms forecasted for at least 0.2 inches of rain. Both the rain gauge and flow meter will be calibrated using standard calibration techniques described in the operation manuals. The purpose for monitoring rainfall amounts and flow rates is to estimate and verify potential flow amounts for various sized storms.

**Figure 5. View of sampling site with rain gauge**


Rainfall Measurements

Rainfall at the site will be measured for each storm event. All data will be recorded by the data logger.

Flow Measurements

Flow measurements during the course of each event will be made using an ISCO 4220 flow meter pressure transducer. The pressure transducer will be mounted within the stormdrain pipe using an ISCO mounting ring. The pressure transducer will be connected to the flow meter which will be powered by an on-site electrical source. The flow meter has an external DC battery as a back up.

Sample Collection

The autosampler holds up to 24 bottles in the chamber. (See Figure 6) A minimum of 8 (5 influent or effluent, 2 field duplicates and 1 field blank), 1000-mL sample bottles will be placed in each autosampler chamber prior to a rain event. Refer to Table 5 for the purpose of each bottle/position in the chamber. The same designation applies to influent and effluent samples.

**Figure 6. View of autosamplers**


**Table 6 Position and Purpose of Sampling Bottles**

| Position/bottle Number | Purpose |
|---|---|
| 1-21 | Bacteria Indicators, pH and conductivity |
| 22 and 23 | Field duplicate- Bacterial indicators, pH and conductivity. |
| 24 | Field Blank |

1. Prior to each rain event the total forecasted duration for the storm will be determined and divided by the number of discrete samples to be collected for each analysis. Total storm duration divided by number of discrete samples will give the time lapse between each sample to be collected. Time interval will be recorded on the Time Interval Log Sheet in Appendix E.
2. At least five discrete influent and effluent samples will be collected according to a pre-programmed time for bacteria indicators, pH and conductivity. Flow-proportional composite sample results will be calculated based on flow levels.
3. Each composite sample will be comprised of a minimum of aliquots from 5 discreet samples including at least 2 samples on the rising limb of the hydrograph, one near the peak, and two on the falling limb.

4. A field blank (sterilized, deionized water) will remain in the samplers for the duration of the event from the time the sterile sample bottles were placed in the automated sampler. Corrective action will be Scott Nolan's responsibility. If de-contamination is necessary, all tubing and connectors will be replaced with clean replacement parts.
5. A single set of duplicate samples will be collected for bacterial indicators, pH and conductivity during each event. Duplicate samples will be collected by installing a clean, sterile split tube which will take an identical sample in two bottles. These samples will be taken at a random point during each event.

## B3 – Sample Handling and Custody

Upon collection, samples will be stored in the refrigerated ISCO samplers at 4 degrees C. At the end of each event the samples will be transported to the Jackson Estuarine Laboratory with ice/ice packs and analyzed within 2 hours. Bacterial samples will be filtered immediately. Samples will be held according to the sample hold times and preservation requirements listed in Table 7. All samples will be labeled for proper identification, including sample identification, date and analysis. The project manager is responsible for the collection, transportation and lab analysis for all samples. Chain-of-custody will be maintained using the Collection Data Log Sheet in Appendix D.

**Table 7 Sample Requirements**

| Analytical parameter | Collection method | Sampling SOP | Sample volume | Container size and type | Preservation requirements | Max. holding time (preparation and analysis) |
|---|---|---|---|---|---|---|
| Conductivity | Auto sampler | User Manual for YSI 33 S-C-T | 100 ml | 1-liter PE | Chilled to <4°C | 28 Days |
| *E.coli*, FC Enterococci | Auto sampler | Appendix A2 | 300 ml | 1-liter PE | Chilled to <4°C | 8 hours* |
| pH | Auto sampler | User Manual for Accumet | 100 ml | 1-liter PE | NA | Analyze Immediately |

*Maximum transport time is 6 hours: must begin analysis within 2 hours after receipt at laboratory.

## B4 – Analytical Methods

The bacterial analyses for this project will be conducted at the UNH/JEL-Microbiology Lab. Analyses include, fecal coliforms, *Escherichia coli* and enterococci membrane filtration methods. The SOPs for the bacterial indicators is included in Appendix A1. The reference limits for each bacterial indicator are listed in Table 8. Steve Jones will be responsible for all corrective actions and will also be responsible for all non-standard method validation.

**Table 8 Water Bacterial Indicators and Reference Limits**

| Indicator | Analytical method SOP Reference | Project Action Level | Analytical/Achievable Method Detection Limit | Project Quantitation Limit |
|---|---|---|---|---|
| *Escherichia coli* | Membrane Filter Procedure, EPA 600/4-85/076; Standard Method 9213D.3 (APHA, 1995) A-1 | NA | 0+ cts/100 mL (depends on dilution and sample volume) | 0+ cts/100 mL (depends on dilution and sample volume) |
| Fecal coliforms | Membrane Filter Procedure, EPA 600/4-85/076 A-1 | NA | 0+ cts/100 mL (depends on dilution and sample volume) | 0+ cts/100 mL (depends on dilution and sample volume) |
| Enterococci | Membrane Filter Procedure, EPA 600/4-85/076; Standard Method 9230C (APHA, 1998) A-1 | NA | 0+ cts/100 mL (depends on dilution and sample volume) | 0+ cts/100 mL (depends on dilution and sample volume) |

## B5 – Quality Control

QC Procedures for Sample Collection

a) Field blanks shall be collected during each event to evaluate whether contamination is introduced during field sampling activities. Sterilized, deionized water shall be placed in sterilized sampler containers and remained uncapped until the end of the rain event. The field blank shall occupy position 24 in both samplers.

b) A minimum of two rounds of equipment blanks shall be conducted. Equipment blanks will be collected once in July and once in October to verify that equipment is not a source of contamination. The blanks will be collected by passing sterile, deionized water through clean equipment and collecting and delivering the samples as "blinds" to the analytical laboratory in the same manner as normal samples.

c) Field duplicates will be collected during each event. Duplicate sample bottles will occupy positions 22 and 23 in each sampler. The duplicate samples will be collected using a split Y tubing device which will place the same sample in two separate bottles. The duplicate samples will be taken during each event but will be taken on a random time basis. The duplicate samples will be compared against themselves but not used as part of the composite sample.

QC Procedures for Bacteria Analyses

Plate counting of bacterial indicators is evaluated by comparing repeated counting of one or more positive samples for this project each month by the same analysts in the laboratory. Replicate counts for the same analyst should agree within 5% and duplicate samples should be within 20%.

Each lot of media is tested using known positive and negative control cultures for each indicator.

Duplicate analyses and sterility tests (filters, pipettes, diluent, graduated cylinders) are performed at least once per test run/batch of samples.

## B6/B7 – Instrument/Equipment Testing, Inspection, Maintenance, Calibration and Frequency

All field equipment will be inspected prior to, and following, each rain event for evidence of damage and/or malfunction. If equipment is not functioning properly, it will be repaired or replaced.

Analytical instrument and equipment testing is performed daily or before each use. pH and Conductivity measurements will be made on all samples at the Jackson Estuarine lab. Included in Table 9 are the equipment to be used during bacterial, pH and conductivity analyses and activities to be accomplished prior to analysis to ensure proper equipment performance.

**Table 9 Instrument Equipment Maintenance, Testing, Inspection and Calibration**

| Equipment name | Activity | Frequency of activity | Acceptance criteria | Corrective action | Person responsible |
|---|---|---|---|---|---|
| Accumet portable pH meter and probe | Calibrate probe with standards | Each use | ±0.01 unit | Correct using manufacturer guidelines | Scott Nolan (JEL-Micro) |
| Lab Line EnviroShaker Model 3597 LB incubator (35°C) | Check temperature | Daily before use and at end of day | Meets analytical requirements | Adjust temperature control and confirm stability of acceptable temperature | Scott Nolan (JEL-Micro) |
| VWR Model 1510 E incubator (41°C) | Check temperature | Daily before use and at end of day | Meets analytical requirements | Adjust temperature control and confirm stability of acceptable temperature | Scott Nolan (JEL-Micro) |
| Fisher Isolatemp Incubator (44.5°C) | Check temperature | Daily before use and at end of day | Meets analytical requirements | Adjust temperature control and confirm stability of acceptable temperature | Scott Nolan (JEL-Micro) |
| Conductivity meter YSI Model 33 S-C-T | Set REDLINE mode control to redline | Each use | Redline adjustment successful | Replace batteries; clean probe | Scott Nolan (JEL-Micro) |
| ISCO Model 6712 refrigerated sampler | Check Temperature | Each Use | Meets analytical requirments | Adjust temperature control | Scott Nolan (JEL-Micro) |
| ISCO 4220 flow meter | Calibrate against calibration meter | Beginning and end of project | ±0.5L/S | Adjust Manning calculation | Scott Nolan (JEL-Micro) |
| ISCO 674 Rain Gauge | Calibrate against other rain gauge | Beginning and end of project | ±0.5 Inches | Send to factory | Scott Nolan (JEL-Micro) |

All balances are serviced by a certified service agency on an annual basis. Deionized water quality is checked during each use by inspection of the in-line conductivity meter.

## B8 – Inspection/Acceptance Requirements for Supplies and Consumables

QC procedures for lab supplies generally follow SM 9020 B.4. All supplies and consumables that are used as part of this project are inspected and deemed acceptable by the person who ordered them. Shipping boxes are opened, the integrity of the contents is determined (broken glass, spillage of powders or liquids, temperature of the material if refrigeration is required) and the quantity of each item ordered is checked against the packing slip. Any discrepancies are noted and the supplier is contacted for replacement of unacceptable items.

All chemicals and microbial media materials are inspected prior to each use. Inspections include confirmation that expiration dates have not been exceeded, and that the physical consistency of materials is not compromised (i.e., fine grained material has not clumped or formed hardened chunks).

All bottles will be sterilized at the Jackson Estuarine Laboratory by Scott Nolan, sealed and kept in clean bags for transportation to and from site.

All calibration tests and results will be documented in the lab book located at the Jackson Estuarine Laboratory.

## B9 – Non-direct Measurements

The project will include use of local weather forecasting information for timing sample collection.

## B10 – Data Management

All data recorded for each sample will be downloaded from the data logger following each storm event. Upon completion of laboratory analysis, sample results are authorized by Steve Jones after being checked for quality control. The project Manager will enter the data into the AbTech Study Database, an Excel Spreadsheet. All results are checked for quality control prior to data analysis and/or reporting. A copy of the original records will be archived at JEL by Steve Jones.

All rainfall data will be downloaded from the data logger following each storm event. Rainfall hyetographs will be developed for each rain event. The hyetograph will show rainfall amounts for the minimum increment of time (15 minutes) recorded by the gauge and a cumulative rainfall curve. A rainfall intensity (inches/hr) curve may also be developed.

All flow data will be downloaded from the data logger following each storm event. A runoff hydrograph will be developed showing flow rates during the monitoring period. A hydrograph will also show the start and end times for the rainfall event.

Datasheets (see Appendices D and E) are used to record information at the time of sampling. These records will be maintained by Scott Nolan. Laboratory analytical results for microbiological parameters are documented in laboratory notebooks and will be entered into a project-dedicated portable computer maintained by Scott Nolan in the JEL-Microbiology Laboratory. Electronic copies of all analytical results are maintained by Scott Nolan.

Databases are maintained on the computer of Scott Nolan. Retrieval of data can be accomplished by opening files on these computers and either printing hard copies or by sending electronic files via email. Copies of SOPs, instrument manuals and other protocols are maintained in 3-ring binders located in each analytical lab and SOPs in electronic files are on the computers of the lab QA Officer. SOPs are reviewed annually or more frequently as changes are required.

All bacterial data and analysis results are maintained and validated by Scott Nolan. Hard copies of raw data are periodically inspected by Steve Jones and Scott Nolan to check for data entry mistakes as well as any possible data analysis problems. Hard copies of datasheets will be kept on file at the Laboratory. Raw data will be kept on disk at the Laboratory. Final products include summary tables and interpretive graphs generated by MS Excel on either PC or Macintosh computers.

## C1 – Assessments and Response Actions

All performance evaluations, audits, data quality assessments, management systems reviews, and technical systems audits associated with the JEL Microbiology Lab are according to those described in Appendix F. The reports will also be reviewed and approved by DES personnel prior to final publication. The response action will be incorporation of valid criticisms and suggestions from these reviewers by the project investigators involved in writing the final report.

Surveillance of data generating activities will also occur. The sample processing activities and microbiological analyses conducted by Scott Nolan will be overseen by Steve Jones and other JEL-Microbiology Lab personnel. Any non-conforming procedures or conditions will be corrected under the supervision of Steve Jones.

**Table 10 Project Assessment Table**

| Assessment Type | Frequency | Person responsible for performing assessment | Person responsible for responding to assessment findings | Person responsible for monitoring effectiveness of corrective actions |
|---|---|---|---|---|
| Field sampling audit | Once at middle of study | Steve Jones JEL | Steve Jones JEL | Steve Jones JEL |
| JEL Microbiology Lab activities | Monthly with sampling | Steve Jones JEL | Steve Jones JEL | Steve Jones JEL |

## C2 – Reports to Management

Scott Nolan is responsible for all report writing and submission to DES. Quarterly reports are required by DES. A final report of the findings will be produced for use by DES and the public. All QA/QC results will be part of the reports to management.For each event the following table will be completed, summarizing rainfall, flow, and influent/effluent pollutant concentrations.

**Table 11 Sampling Event Summary**

| Event | Start Date/Time | End Date/Time | Max. Hourly Rainfall Intensity ($ft^3$) | Runoff Volume Through Device ($ft^3$) | EMC: *Escherichia coli* (cfu/100mL unless otherwise noted) | | EMC: Fecal coliforms (cfu/100mL unless otherwise noted) | | EMC: Enterococci (cfu/100mL unless otherwise noted) | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Influent | Effluent | Influent | Effluent | Influent | Effluent |
| 1 | | | | | | | | | | |
| 2 | | | | | | | | | | |
| 3…15 | | | | | | | | | | |

Two critical reporting calculations will be load reduction and concentration efficiency ratio.

Load Reduction Ratio (all events): For each pollutant constituent (bacterial indicators) a summation of the loads calculation will be made. The load reduction efficiency of the system will be based upon the sum of the total outlet loads to the sum of the total inlet loads for all events as follows:

%Load Reduction Efficiency = 100*(1-(A/B))

Where:

A = Sum of Effluent Load

B = Sum of Influent Load

n = number of qualified events

See ETV Draft 4.1 http://www.epa.gov/etv/pdfs/vp/04_vp_stormwater.pdf

Concentration Efficiency Ratio (single event): For each pollutant constituent (bacterial indicators) and storm event, an efficiency ratio (ER) will be calculated based on the reduction in the event mean concentration (EMC) from the influent to the effluent. The efficiency ratio will be calculated and reported for each storm event as follows:

Efficiency Ratio = 100*(1-(effluent EMC)/(influent EMC)

## D1 – Data Review, Verification and Validation

A review of all bacterial and other data generated by this project will be conducted by Scott Nolan. The completeness, transcription errors and compliance with procedures will be evaluated by comparison of tabulated results to what has been proposed in the original project proposal and this QAPP. The specific activities include the generation of data including, flow measurements, precipitation amounts, duration of the events, and bacterial indicators, pH and conductivity. Omissions of data in spreadsheets will trigger a search of raw datasheets for missing data or possibly reanalysis of the questionable sample, if possible. If reanalysis is not possible or if data remain missing, invalid or otherwise affected entries will not be incorporated into the useable data set. When results appear to be abnormal, all appropriate project participants will review the available data and discuss the problem in periodic meetings to attempt to identify potential problems in sampling or analyses.

Collection Data Log Sheets will be reviewed to determine adherence to chain-of-custody and sample holding times. Chain-of-custody documentation will be maintained on the Collection Data Log Sheets (Appendix D).

## D2 – Verification and Validation Procedures

Initial data review is conducted by Scott Nolan, the initial recipient of data and the generator of bacterial data. Databases will be inspected for missing data, repetitive data entries, other incorrect data entries, and measurement performance criteria for bacterial indicators (Table 4), etc. Corrective action will include going back to original datasheets, forms and lab books to check numbers against those in compiled databases. The validated data are entered into databases, and re-validated by Scott Nolan. Printed hard copies of summarized data are then reviewed by both Scott Nolan and Steve Jones (bacteria only). Inconsistencies and incompleteness are discussed and corrective actions are taken by Scott Nolan. Steve Jones will inform Scott Nolan of any QC problems that arise in the JEL Microbiology Lab prior to any analysis of samples. There are no project acceptance limits or other data criteria.

## D3 – Reconciliation with User Requirements

The quarterly reports will be the mechanism by which data users (DES) will be able to have input on the results. In compiling the reports, Scott Nolan will be able to assess anomalies or departures from assumptions. The sample design is quite simple and the only anticipated problem might be incompleteness of the data, which should not be a problem. Departures from assumptions about contaminant concentrations in effluent would be difficult to determine as there is little equivalent information available for comparison. The eventual use of the data as baseline information for the potential decrease in bacterial loading to NH estuarine waters by stormwater will be discussed in meetings by the NHEP Technical Advisory Committee, the NHEP Water Quality team and by DES coastal water quality and Shellfish Program personnel. All of these discussions are anticipated to occur both during the project and after the final report is written and submitted.

## References

American Public Health Association. (APHA). 1998. Standard Methods for the Examination of Water and Wastewater. 20th Edition. American Public Health Association, Washington, DC.

Jones, S.H. 2003. Tracking bacterial pollution sources in stormwater pipes. Final report. New Hampshire Estuaries Project, Portsmouth, NH.

Jones, S.H. (ed.). 2001. A Technical Characterization of Estuarine and Coastal New Hampshire. New Hampshire Estuaries Project, Portsmouth, NH.

Jones, S.H. and N. Landry. 2003. Tracking bacterial pollution sources in Hampton Harbor. Final report. New Hampshire Estuaries Project, Portsmouth, NH.

Jones, S.H. and R. Langan. 1994. Land use impacts on nonpoint source pollution in coastal New Hampshire. Final Report. NH Office of State Planning/Coastal Program, Concord, NH. 103 pp.

Nash, C. 2002. New Hampshire Department of Environmental Services Shellfish Program: 2001 annual report. NH Department of Environmental Services, Concord, NH.

Trowbridge, P. 2003. Field evaluation of wet weather bacteria loading in Hampton/Seabrook Harbor. Final Report. . New Hampshire Estuaries Project, Portsmouth, NH.

U.S. Environmental Protection Agency (USEPA). 1986. Test methods for *Escherichia coli* and enterococci by the membrane filtration procedure. EPA 600/4-85/076. Environmental Protection Agency, Environmental Monitoring and Support Laboratory, Cincinnati, OH.

US EPA Draft Verification Protocol for Stormwater Source Area Treatment Technologies Draft 4.1, (ETV Verification Protocol Draft 4.1 March 2002). Online at http://www.epa.gov/etv/pdfs/vp/04_vp_stormwater.pdf

# Appendix A1

## Standard Procedure for Detection of Total Coliforms, Fecal coliforms, *Escherichia coli* and Enterococci from Environmental Samples

September, 2002

Latest Revision

May, 2003

Prepared by:
**Stephen H. Jones & Tamara Bryant**
Jackson Estuarine Laboratory
University of New Hampshire
Durham, NH 03824

| | |
|---|---|
| **Stephen Jones Date** | **Tamara Bryant Date** |
| **Author, Lab Director and QA Officer** | **Co-Author** |

# Table of Contents

# INTRODUCTION

Various bacterial species and groups of bacteria have been used as indicators of fecal contamination in surface water, groundwater and food. In New Hampshire, state laws dictate the use of 4 different bacterial indicators for use for classifying different types of water. Total coliforms are used for groundwater and some waste water treatment facility (WWTF) permitted discharges, fecal coliforms are used by the NH Shellfish Program for classifying shellfish harvesting areas, enterococci are used for classifying recreational marine and estuarine waters and *Escherichia coli* is used for freshwater recreational waters. The microbiology lab at the Jackson Estuarine Laboratory has conducted projects and has otherwise worked closely with various state agencies concerned with surface water quality in the Seacoast region of NH. Protocols have been used and modified over the past 15 years for the detection and enumeration of different bacterial indicators of fecal contamination. The most recent protocols are presented in the following sections.

This Standard Operating Procedure also includes descriptions of sampling and media preparation. The basic approach is to collect water samples in sterile containers from the field and transport them on ice to the lab as soon as possible. The water samples are filtered through membrane filters and the organisms caught on the filters are grown to colonies on indicator specific media and conditions. The colonies showing the indicator-specific reaction on the agar media are enumerated following appropriate incubation times.

## I. Space Requirements

### 1.1 Specimen Collection.

Not applicable.

### 1.2 Specimen Intake, Processing and Detection.

This area should include 2 meters of counter space with shelves for storage, and equipped with a water source and a refrigerator. A small area must be designated “clean” for paper work for the prevention of contamination to yourself and others.

### 1.3 Biochemical Preparation.

This area should include approximately 3.5 meters of counter space with shelves, a readily available de-ionized water supply, an autoclave, storage for biohazard waste, and a large sink.

## II. Equipment Requirements

### 2.1 Specimen Collection.

Laboratory van and/or boat for access to sites, devices for reaching and sampling from surface water.

### 2.2 Specimen Intake, Processing and Detection.

Autoclave, balance, vacuum pump, filter towers, vortex, Stomacher, computer system for database management, printer, 44.5•C incubator, 35•C incubator, 41•C incubator, thermometers, 4•C refrigerator, ice chest, alcohol burners, loops, scissors, forceps, pipette pump.

### 2.3 Biochemical Preparation.

Autoclave, test tube washer, hot plate stirrers, alcohol burners, 4•C refrigerator, -22•C freezer, Parafilm®, balance, vacuum pump, filter towers, filter membranes, vortex, pH meter.

## III. Chemicals and Supply Requirements

### 3.1 Specimen Collection.

1000 ml sterile Whirlpac® bags, or autoclavable plastic bottles, waterproof gloves, sterile gloves, permanent marker, cooler and ice, datasheets.

### 3.2 Specimen Intake, Processing and Detection.

filter membranes, cellulose pads, Buffered peptone water, de-ionized water (DI) DEPC treated DI, goggles, sterile gloves, pipettes of various volumes, graduated cylinders, sterile cellulose pads, Petri dishes containing agar media,

### 3.3 Biochemical Preparation.

Autoclavable flasks (25 ml- 4000 ml), beakers (10 ml- 500 ml), test tube racks, 50 ml test tube with caps, 13 ml test tubes with caps, stir bars, 15 mm Petri dishes, 3 mm Petri dishes, weigh boats, 0-10 µl pipette,10-100 µl pipette, 100-1000 µl pipette, 1 ml-10 ml pipette, pipette tips for each size pipette, autoclave tape, aluminum foil, indole, mTEC, Mac Conkey, Oxidase, Tryptic Soy agar, Tryptic Soy Broth, Simmon's Citrate, Urea Agar, Urease, Methyl Red, Voges-Proskaur, DEPC treated de-ionized water.

## IV. Biochemical Media, Solutions, Preparation and Storage

### 4.1 Media

All media is to be prepared in a sterile fashion under a hood, lightly covered with tin foil or foam stoppers, wearing gloves, lab coat, autoclave mitts, goggles and tie backs for those with long hair. Store the media agar side up to prevent condensation and at 4•C in plastic sleeves (Atlas and Parks, 1993).

#### 4.1.1 Mac Conkey Agar (Mac)

50 g of Mac Conkey
1000 ml DEPC DI
Mix and boil to dissolve
Autoclave
Dispense to small plates flaming the lip of the flask between plates.

**4.1.2 mTec Agar**

45.3 g mTec agar
1000 ml DI
Mix and boil to dissolve
Autoclave
Dispense to small plates flaming the lip of flask between plates.

**4.1.3 Simmon's Citrate (SimCit)**

24.2 g of Simmons Citrate
1000 ml DEPC DI
Mix and boil to dissolve
Autoclave
Dispense to small plates flaming the lip of the flask between plates.

**4.1.4 Tryptic Soy Agar (TSA)**

40 g Granulated TSA Agar
1000 ml DI
Mix and boil to dissolve
Autoclave
Pour to large plates flaming the lip of the flask between plates.

**4.1.5 Urea Agar**

29 g Urea Agar Base (in 5ºC)
100 ml DEPC DI
Filter sterilize/DO NOT HEAT
*In **separate flask** suspend:*
15 g Granulated Agar
900 ml DI
Autoclave/Cool to 55ºC
Add Filtered Urea Agar Base
Mix well and pour into small plates flaming the lip of the flask between plates.

**4.2 Solutions**

All solutions are to be prepared in a sterile fashion under the a hood, wearing gloves, lab coat, goggles, autoclave mitts and tie backs for those with long hair (Atlas and Parks, 1993).

**4.2.1 Buffered Peptone Water (BPW)**

2.8 g Na2HPO4 (Sodium Phosphate Dibasic)
1.2 g KH2PO4 (Potassium phosphate Monobasic)

4.0 g NaCl
8.0 g Bacto peptone
800 ml DEPC DI
Adjust pH to 7.2 with HCl
Dispense 9.6 ml into large tubes and cap
Autoclave
Store at 4•C

**4.2.2 Brain Heart Infusion Broth**

37 g Dehydrated Brain Heart Infusion Powder
1000 ml DI
Adjust pH to 7.4±.02
Dispense 10 ml into 20 ml tubes
Cap and Autoclave
Remove and cool to room temperature then store at 4ºC

**4.2.3 EC MUG**

29.68 g Dehydrated EC medium with MUG
800 ml DI
Adjust pH to 6.9± .2
Carefully dispense 10 ml in to 20 ml tubes containing inverted Durham tubes
Remove and cool to room temperature then store at 4ºC

**4.2.4 Indole Reagent**

75 ml Iso-Amyl Alcohol
25 ml conc. HCl
pH to <6.0 then add:
5 g p-dimethylaminobenzadehyde
Store at 4ºC

**4.2.5 LT Broth**

28.48 g Dehydrated lauryl tryptose broth
800 ml DI
Warm to dissolve
Adjust pH to 6.8 ± .0
Dispense 10 ml into 20 ml tubes containing inverted Durham tubes
Autoclave
Store at 4ºC

**4.2.6 MRVP Broth (Methyl-Red, Voges-Proskauer)**

5.0 g Glucose
5.0 g K2HPO4
3.5 g Pancreatic digest of casein
3.5 g Peptic digest of animal tissue

Add all components to 900 ml of DI.
Mix to dissolve
Bring to 1000 ml
pH to 6.9 at 25ºC
Distribute 10 mls into 50 ml tubes and cap
Autoclave
Store at 4ºC

**4.2.7 MRVP Indicator Solution**

0.1 g Methyl red
300 ml 95 % Ethyl alcohol
Bring to 500 ml with DI
Filter sterilize
Store at 4ºC

**4.2.8 Oxidase Reagent 1%**

1 g Tetramethyl-p-phenylenediamine dihydrochloride
100 ml DI
Filter sterilize
Store in dark area at 4ºC

**4.2.9 Tryptic Broth for Indole**

80 g Tryptic Soy Broth
1000 ml DI
Warm to dissolve
Dispense 5 mls to small tubes and cap
Autoclave
Store at 4ºC

**4.2.10 Urea substrate (for use with mTEC)**

4 g Urea pellets
200 ml DI
0.02 g Phenol Red Indicator
Mix to dissolve
Adjust pH to 5.0 with dilute HCl (10%)
Filter sterilize
*DO NOT AUTOCLAVE*
Store at 4ºC

**4.2.11 Voges-Proskauer Indicators**

Difco VP-A # 261192
Difco VP-B # 261193
Use per manufacturers instructions

**4.2.12 Cryoprotectant**

Solution 1:
8.5 g NaCl
0.65 g potassium phosphate dibasic
0.35 g potassium phosphate monobasic
1000 ml DI
Autoclave and cool to room temperature
Solution 2:
50 ml autoclaved glycerol, cooled to room temperature
50 ml DMSO
**Aseptically** mix 800 ml of Solution 1 to all of Solution 2
Store at 4ºC

***Hints***
When boiling any agar media it is wise to keep an eye on the foam that forms on the surface of the media. As the temperature increases in the flask the foam rises (Atlas and Parks, 1993). When the foam is one inch thick quickly remove the flask from the stir plate. This will prevent the media from boiling over. Put the media in the autoclave as soon as possible to prevent premature setting.

# V. Specimen Collection

## 5.1 Water Samples

With a gloved hand, submerge 100 ml Whirlpac® bag 10-30 cm below the water surface in a direction facing the current and open. For plastic bottles, submerge the bottle with gloved hand in a direction facing the current and remove cap. In a boat, sample from the upstream side. Care must be taken to avoid disturbance of the surrounding waters prior to or during the sample retrieval. Fill the bag or bottle to capacity and twist the bag closed or re-cap the bottle before surfacing**.** The data sheet should be made of Write-In-the Rain® paper, filled out completely with a Write-In-the Rain® marker and those spaces not applicable crossed off. Record the time**,** date**,** conditions**,** and collector's initials. Put sample on ice and transport.

## 5.2 Finding and Identifying Scat

There are general approaches to locating scat, and the details of the method used are presented in the NHDES SOP for identification and collection of scat samples (Appendix 2). Knowing the type of habitat that a certain animal resides is critical. A large broad sweep of a field and the surrounding transitional zone is an excellent place to start. Riparian zones often provide a wide variety of scat. Try to identify paths to water and food sources. Temporal bodies of water offer seasonal scat collection. One must also remember that some animals mark territory by defecating or urinating on conspecific scat. A witnessed event is the best identification, but in the wild very rare. Identification of scat can be assisted with the aid of guide books.

## 5.3 Fecal Samples

Fecal samples should be collected fresh, this reduces the chance of contamination, resource competition, and transformation. Samples that are very dry, found after a rain event, or that show signs of deterioration should not be collected.

Invert Whirlpac® bag over gloved hand and pick up quantity of ***fresh*** fecal specimen. Make sure the sample is as debris free as possible. Revert bag over hand and feces, twist shut. The data sheet should be made of Write-In-the Rain® paper, filled out completely with a permanent marker and those spaces not applicable crossed off. Record date, time, sex (if possible), location, species/breed (using a species code list, appendix 2) and the collectors' initials. Put sample on ice for transport and processing.

### 5.4 Preparation for incoming fecal and water samples.

Prior to receiving the samples the area should be disinfected. The log book with date and time of sample arrival should be ready for entries. Check the samples against the original collection sheets making sure that all samples have the correct information on their respective containers. Record the samples and their conditions into the log book and have the person delivering the specimens sign the book.

## VI. Specimen Intake

### 6.1 Acceptable Samples

Samples of water should be in water tight containers preferably in a secondary sealed plastic bag. Containers should be labeled with time and date of sample collection, site number and sample collector's name. The water samples have to be analyzed within 2 hours of receipt in the lab. If this holding time is exceeded, then any data for analysis of such samples need to be "flagged", or labeled in a way to reflect this violation of sample integrity.

Fecal samples should be fresh in nature with minimal debris attached. If it appears that a sample has been compromised or has compromised others during transport it/they should be discarded. It is important to note that the integrity and homogeneity of the samples should be without question. A customized Laboratory Management System (see 6.2) should be in place to track samples and analytical data. These data may include: Sample number that is unique to that site, date received, sample descriptions, additional comments, notations about special handling, and name of person delivering samples.

### 6.2 Specimen Sample Log Sheet

A log book of collection sites, dates the site was sampled, the type of specimen collected, and the date and time of receipt of the sample in the lab should be maintained. Two copies for each sample is recommended. A log book of samples received into the lab and the condition of the samples should also be maintained. A spreadsheet database should be utilized for tracking the specimen and its isolates through the laboratory procedures.

Occasionally, sample analysis requires use of chain of custody sheets for some clients. The procedure is to sign the sheets as required and to take a copy for our laboratory records.

### 6.2.1 Sample Log Sheet

**COLLECTION DATA LOG SHEET**

Site Name:
Type of Sample
Site Description:
Fecal
Water
Animal Species:
Location:
Water Temp:
% DO Saturation:

DO:
pH:
Conductivity:
Location:
In stream
Seep
Swale
Storm Drain
Other:
Street:
Town:
Watershed:
Date:
Time:

Sampled by:

Parameters
Weather:
Air Temp:

Flow Rate:
Comments and Sketch/Description

Delivered to lab by:

Date:
Time:
Received by:

# VII. Detection and Biochemical Confirmation Methods

## 7.1 Water Samples

Use flame sterilize forceps dipped into alcohol to aseptically place a sterile gridded 0.45 µm membrane filter on the filter base of a sterile 250 ml filter and attach magnetic filter tower. Vigorously shake the sample bottle or bag at least 30 x and measure out volume to be filtered either in a sterile graduated cylinder or by using a sterile pipette. If the sample is turbid or is suspected of having a high colony count, dilutions of a water sample may be necessary. Add one ml sample to 9 ml sterile BPW and decimally dilute from $10^{-1}$ to $10^{-7}$. Pour up to 100 ml of a sample into the filter tower and conduct routine filtration at 25 millibar until all water has passed through the filter. Turn the vacuum pump off and aseptically remove the filter using sterile forceps.

Positive and negative samples are to be run with each sample set. These include positive samples of enterococci, total coliforms, fecal coliform and *E. coli,* and negative samples for total and fecal coliforms and enterococci. If the results of the positive or negative controls indicate either contamination or culture problems, all sample results will be discarded and samples will be reanalyzed, if holding time requirements are not exceeded.

Field duplicates are routinely collected as part of projects. Colony counts of positive field samples, as well as laboratory duplicate analyses, are expected to agree within 5%.

Each quantification procedure for the different bacterial indicators has specific verification procedures that are followed, and the procedures used at JEL are exactly as described in Standard Methods (APHA 1998). Counts are then adjusted based on the percent verification of these results. Membrane filtration methods require monthly verification of the identity of 10 colonies from one positive sample, as well as representative colonies of non-positive colonies. All positive and negative total coliforms, fecal coliforms and *E. coli* colonies are verified by inoculation of LT and EC-MUG broths to check for lactose fermentation at 35°C, lactose fermentation at 45°C and ß-glucuronidase activity. For enterococci verification, the colonies are streaked to BHI agar, growth is transferred to BHI broth. The 24 h suspension is tested for catalase activity using $H_2O_2$ and checked microscopically for cocci and gram stain. Catalase negative, gram positive cocci cultures are then transferred to bile esculin agar (35°C), BHI broth (44.5°C) and BHI broth + 6.5%NaCl (35°C) to verify cultures as fecal streptococci and enterococci.

### 7.1.1 Detection of Total Coliforms

Place the filter onto an M-Endo medium plate, grid side up, by rolling the paper onto the agar surface to minimize air bubbles under the filter. Incubate all plates inverted in incubators at 35±0.5 °C for 22-24 hours (APHA, 1998). Count the colonies that are pink to dark-red and have a metallic surface sheen for each sample/site at best (or all) dilutions (10-30 readable colonies) and record as total coliforms.

Pick an isolated colony from a plate from each sample batch and inoculate **Mac Conkey agar**, **Trypticase Broth for indole**, **Urea agar**, **MRVP Broth** and **Simmons Citrate agar,** incubate at 35±0.5 º C overnight. Conduct **Oxidase** test (looking for no color change) on isolates that are **Citrate negative** (no growth, no color change), **Urea negative** (no color change), and **Indole positive** (pink to violet color), **MRVP** (red color change), **Mac Conkey positive** (pink colonies). These are confirmed *E. coli* colonies, the target species for total coliform analyses.

### 7.1.2 Detection of Fecal Coliforms and *E. coli*

Place the filter onto an **mTEC** medium plate, grid side up, by rolling the paper onto the agar surface to minimize air bubbles under the filter. Incubate all plates inverted in incubators at 35±0.5 °C for 2 h and at 44.5±0.2 °C for 22 hours (USEPA, 1986).

Count the yellow colonies for each sample/site at best (or all) dilutions (10-30 readable colonies) and record as fecal coliforms (Rippey et al., 1986). Remove top of Petri dish and invert onto counter. Place cellulose pad in lid and pipette 2.0 ml of **Urea substrate solution** onto pad. Roll filter onto pad to discourage air bubbles, cover and incubate for 10-20 minutes at room temperature. Count the yellow/yellow brown colonies using a magnifying lens and record as *E. coli*.

Pick an isolated colony from a plate from each sample batch and inoculate **Mac Conkey agar**, **Trypticase Broth for indole**, **Urea agar**, **MRVP Broth** and **Simmons Citrate agar,** incubate at 35±0.5 º C overnight. Conduct **Oxidase** test (looking for no color change) on isolates that are **Citrate negative** (no growth, no color change), **Urea negative** (no color change), and **Indole positive** (pink to violet color), **MRVP** (red color change), **Mac Conkey positive** (pink colonies). These are confirmed *E. coli* colonies.

For ribotyping projects, pick up to ten isolated, removable, presumptive *E. coli* colonies (yellow colonies after Urease test) per plate and four quadrant streak to **Tryptic Soy Agar**. Incubate at 35±0.5 °C for 24 hours. Repeat the biochemical tests for confirmation of *E. coli* colonies. Those that meet the above criteria can be re-streaked to **TSA** and incubated at room temperature overnight. Keep presumptive *E. coli* isolates frozen in a **Saline Phosphate Buffer and Cryoprotectant** media at -80°C.

### 7.1.3 Detection of Enterococci

Place the filter onto an **mE** medium plate, grid side up, by rolling the paper onto the agar surface to minimize air bubbles under the filter. Incubate all plates inverted in an incubator at 41±0.5 °C for 48 h (USEPA, 1986). Transfer membrane filter to the surface of an EIA agar plate and incubate at 41±0.5 °C for 20 min. Count pink-red colonies that form a black to reddish-brown precipitate in the agar below the colony using a magnifying lens and record as enterococci.

Pick an isolated colony from a plate from each sample batch and inoculate **Brain Heart Infusion (BHI) agar**, incubate at 35±0.5 º C overnight. Conduct a catalase and a gram stain test on an isolated colony. For catalase negative/gram positive cultures, transfer a colony to BHI broth and incubate for 24 h at 35±0.5 °C. Inoculate BHI broth (incubate at 45±0.5 °C for 48 h),

BHI broth with 6.5% NaCl (incubate at 35±0.5 °C for 48 h) and streak a plate of bile esculin agar incubate at 35±0.5 °C for 48 h). Growth on both media indicates that the colony belonged to the enterococcus group of the fecal streptococci.

### 7.2 Detection of *E. coli* in Fecal Samples

For fecal samples, add 1 g of feces to 9 ml of **BPW** in a sterile Whirlpac® and place in stomacher on medium for 30 sec. Using 2.5 mls of digest, serial dilute in **BPW** to 10-7. Make sure that each tube is labeled as to the dilution, this reduces error.

Filter 10 mls of all dilutions (except first) of every sample and place on **mTEC agar** that has been labeled with the appropriate dilution. Incubate at 44.5°C for 24 hours.

Count and record yellow colonies for each sample/site at best dilutions (10-30 readable colonies).

Remove top of Petri dish and invert onto counter. Place cellulose pad in lid and pipette 2.0 ml of **Urea substrate solution** onto pad. Place filter colony side up onto pad, cover and incubate for 10 minutes at room temperature. Count and record yellow colonies.

Pick up to ten isolated, removable, presumptive *E. coli* colonies (yellow colonies after Urease test) per plate and 4 quadrant streak each onto separate **Tryptic Soy Agar** plates.
**Incubate** at 35-37°C for 24 hours.

Pick one isolated colony from each plate and inoculate **Mac Conkey agar, Trypticase Broth for Indole**, **Urea agar, MRVP Broth** and **Simmons Citrate agar**, incubate at 35-37º C overnight.

Conduct **Oxidase** test (looking for no color change) on isolates that are **Citrate negative** (no growth, no color change), **Urea negative** (no color change), and **Indole positive** (violet color), **MRVP positive**, **Mac Conkey positive** (pink colonies). Those that meet the above criteria can be re-streaked to **TSA** and incubated at room temperature overnight.

Keep presumptive *E. coli* isolates frozen in a **Saline Phosphate Buffer and Cryoprotectant** media at -80°C.

### 7.3 Storage of all bacteria

From **TSA plate** pick one colony and place in to vial. Add 1.0 ml of **buffer/ protectant** mixture. Vortex until colony is dispersed completely in buffer. Label cap with specimen number and original collection date. Record the tray and shelf number into the log book then enter it to the database. Place in labeled cryo-rack and put in -80º C freezer.

## VIII. Notes on Quality Control

The JEL Microbiology Laboratory QA Plan provides details of QA procedures required to detection of bacterial indicators. The notes below are additional details specific to these procedures.

## 8.1 General Laboratory Practices

The first concern of any lab is the safety of its personnel. Each person working in the laboratory is trained in lab safety and will be well informed of any hazardous material they might encounter. A chemical roster is stored in the laboratory and Material Safety Data Sheet (MSDS) folders are stored in the JEL Lab Technician's office and kept up to date. Gloves, goggles, gowns or lab coats are advised. No open toed shoes or shorts are allowed. Personnel that have long hair need to tie it back to prevent injury. All instrumentation, cold units, pipettes, incubators, etc. are routinely calibrated by a qualified instrumentation technician.

## 8.2 Specimen Collection

*All collection devises and receptacles must be sterile*. Gloves should be rinsed (water) or changed (feces samples) between each sample collected. If a spatula or other collection devise is used it must be sterile. Feces may be double bagged to insure no contact. Water sample lids should be tightened and each bag/ bottle stored and transported upright. Leaking specimens and others in the same transport container may be cross contaminated and should not be accepted. Care should be taken that no specimen comes in direct contact with any other. If at any time a question of contamination arises, discard the sample.

## 8.3 Specimen Intake and Processing

The laboratory bench surfaces and instruments are to be decontaminated and or autoclaved prior to introduction of specimens. A daily log of instrument cleaning, and temperature control should be checked off, initialed and displayed in a prominent place. If a specimen has been spilled use the lab approved spill kit and all precautions to prevent contamination. Change pipette tips, forceps, and filter towers after each specimen serial dilution.

## 8.4 Biochemical Preparation and Detection

Biochemicals are the foundation of accurate indicator identification. If the methods or materials are compromised the results would be in question. Gloves and goggles need to be worn for safety and the reduction of contamination. Those that have long hair should tie it away from the face. Compounds, chemicals and other disposables that are received at the lab should have the receive date and the date opened recorded on the receptacle. It is recommended that media and solutions be made in autoclaved containers, under the hood and autoclaved unless otherwise stated. All disposables should be aliquoted to the appropriate containers. Storage of the disposables described in the media section should be strictly followed. The date and the initials of the person that made the disposable should be clearly written on the container. A weekly check of the plated media and a day-of-analysis aseptic check of the pH of solutions is required. As always use the oldest acceptable media first. Tubes and other glass and plastic ware (pipette tips, graduated cylinders) should be capped, autoclaved and stored in the autoclave bags.

References

American Public Health Association. (APHA). 1998. Standard Methods for the Examination of Water and Wastewater. 20th Edition. American Public Health Association, Washington, DC.

Atlas, R., L.C. Parks, Eds. 1993. Handbook of Microbiological Media. CRC Press. Boca Raton, FL.

Rippey, S.R., W.N. Adams and W.D. Watkins. 1987. Enumeration of fecal coliforms and *E. coli* in marine and estuarine waters: an alternative to the APHA-MPN approach. J. Wat. Pollut. Cont. Fed. 59: 795-798.

U.S. Environmental Protection Agency (USEPA). 1986. Test methods for *Escherichia coli* and enterococci by the membrane filtration procedure. EPA 600/4-85/076. Environmental Protection Agency, Environmental Monitoring and Support Laboratory, Cincinnati, OH.

## Appendix A2

SOP for Automated Sample Collection of Bacterial Indicators

1. Sterilize ISCO automated sampler bottles and caps in autoclave.
2. Label each bottle as sample, duplicate or blank.
3. Fill the field blank bottles with sterilize, deionized water.
4. Place sterile bottles in the ISCO bottle array and place array in sampler.
5. Once the sampler is triggered, lines will purge by drawing water to the manifold, purging and finally drawing in the sample all the way into the sample bottle.
6. After the event, open the sampler and place the sterile caps on the bottles.
7. Complete the labels by adding site identification, date, and time of sample collection.
8. Place the bottles in a cooler on ice or ice packs.
9. Complete the collection data log sheet.
10. Download all data from ISCO flow meter.
11. Transport to laboratory within the established holding times.

## Appendix B

## AbTech's Smart Sponge®

Surface Water Quality Solutions For Clean Water Today and Tomorrow

AbTech Industries, Inc. has developed a patented technology over the past seven years based on a proprietary blend of synthetic polymers aimed at removal of hydrocarbons and oil derivatives from surface water. AbTech's process creates a porous structure (see Figure A) with hydrophobic and oleophilic characteristics capable of selectively removing hydrocarbons while allowing high flow rates. This structure is highly porous; as hydrocarbons are absorbed into its structure, the Smart Sponge® swells and maintains porosity and filtering capabilities.

### Smart Sponge® Plus

Over the past 18 months, AbTech Industries has worked on the development of a new solution capable of treating microorganisms as well as hydrocarbons named Smart Sponge® Plus. AbTech has developed a technology capable of binding an Antimicrobial Agent to its proprietary polymers thereby modifying their surface and adding micro biostatic features while maintaining the oil absorbing capabilities (see Figure B).
The Agent used for this innovative technology is an Organosilane derivative (see its chemistry in Figure C), which is widely used in a variety of fields including medical, consumables, pool equipment and consumer goods.

:

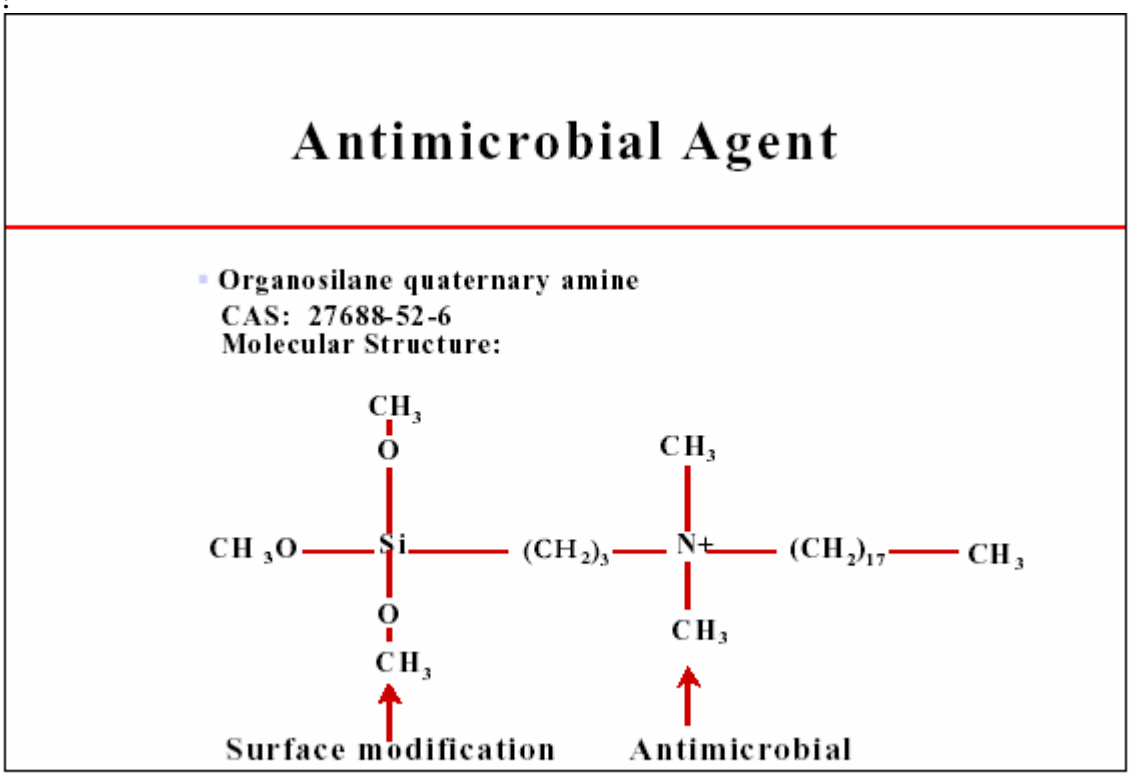



Figure C

Its mode of action is very simple (no Chlorine or heavy metals involved) and - in surface-bound applications – it neither introduces chemicals into the treated water nor produces toxic metabolites. Its chemical structure ensures long-term stability to natural agents and reduced degradation. This Antimicrobial Agent is registered with EPA for various applications and has been proven successful in those applications against several microorganisms (see Figure D).

In the Smart Sponge® Plus, the Antimicrobial Agent is chemically and permanently bound to the polymer surface and it does not leach or leak, therefore avoiding any downstream toxicity issues. The antimicrobial mechanism is based on the Agent's electromagnetic interaction with the microorganism cell membrane, causing the microorganism disruption (see Figure E), but no chemical or physical change in the agent. Antimicrobial activity does not reduce the agent capability or cause its depletion and, therefore, maintains long-term effectiveness.

**Tested Positively on:**

- Aspergillus Niger
- Trychophyton Mentagrophytes
- Penicillium Pinophilum
- Chaetomium Globosum
- Trichoderma Virens
- Aureobadisium Pullulans
- Staphylococcus Aureus
- Escherichia Coli
- Pseudomonas Aeruginosa
- Candida Albicans
- Salmonella
- Klebsiella Pneumoniae

Figure D

Smart Sponge® Plus internal laboratory efficiency testing has verified its micro biostatic effectiveness with E.coli. Additional internal laboratory testing in dynamic settings (in-line filtration) was performed in order to simulate stormwater runoff conditions. Figure G outlines the microbial reduction capabilities of the Smart Sponge Plus with Staphilococcus Aureus and E.coli.

Smart Sponge® Plus will also perform as a fungi static, odor and mildew control and will be featured in existing and future UUF-Plus catch basin inserts for stormwater runoff treatment.

4110 North Scottsdale Road, Suite 235
Scottsdale AZ 85251
480-874-4000 or 800-545-8999
FAX: 480-970-1665
www.abtechindustries.com

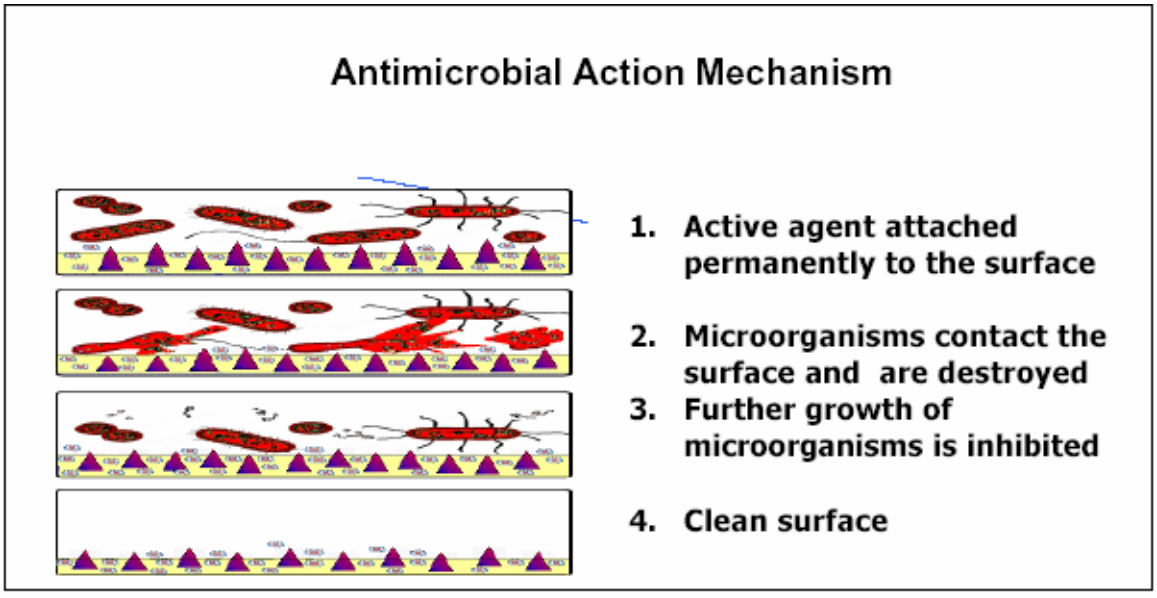



Figure E

Figure G

# Appendix C

Using Autosamplers for Sampling Storm Events
Steve Roy, P.E. Burlington, Vermont Public Works

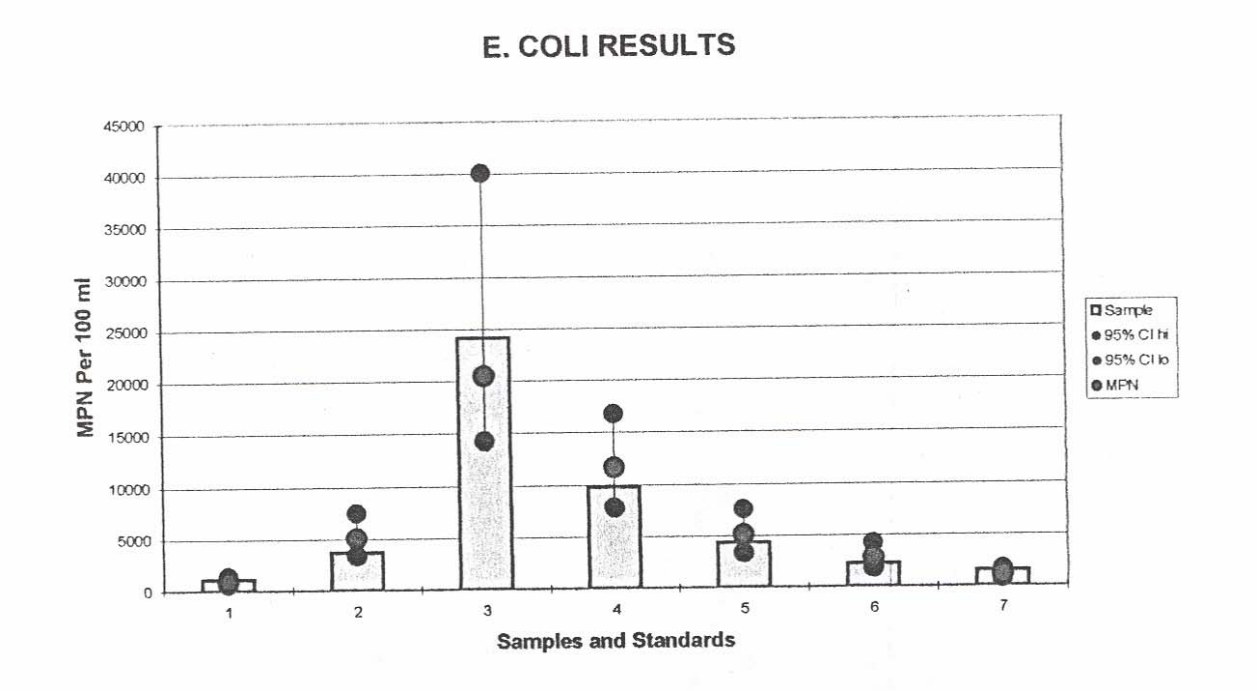
A stock solution was prepared containing a relatively known concentration of *E. coli* bacteria. This was accomplished by using the wastewater plant's primary effiuent as a bacteria source. Previous tests had revealed primary effluent bacteria concentrations around 2.5 million per 100 mls. The sample was blended to obtain a homogeneous mixture.

The above stock solution was used to prepare seven (7) standards with varying *E. coli* concentrations. Since bacterial loading in storm water tend to peak quickly, we started with a relatively low background concentration working up to a peak at the third standard and gradually tapering of background levels. These standards were made at the laboratory in 1 gallon sterile containers and were sampled at the gauging station just prior to running the test. Also, two (2) containers each with sterile deionized water were run through the autosampler for before and after the test to determine pre- and post- bacteria concentrations. These tests yielded results of 50 and 261 MPN (most probable number) per 100 ml of *E. coli,* respectively.

At the station, an extension was placed on the existing sample hose to facilitate placement of the tubing in each respective solution. The ISCO 3700 was programmed to provide the following steps for each sample cycle: a) purge sample line, b) draw liquid to pump head, c) purge sample line, d) take sample, e) purge sample line. While this procedure helps prevent cross-contamination of samples in the field, it presented minor problems for this pilot study. Since we didn't want the purge cycles to contaminate our standards, we prevented the sampler from purging into the standards. Therefore, a person at the sample hose removed the end out of each standard during purge cycles.

The first sample drawn was sterile deionized water to determine what bacteria concentration, if any, was in the sampler tubing. Standards 1 through 7 were then drawn in order, followed by the post-test sterile deionized water to determine if bacteria was left in the tubing. All samples were brought back to the laboratory and immediately analyzed for *E. coli* using the IDEXX Quantitray 2000 method with duplicates of each dilution.

TEST RESULTS:
The graph on the next page shows results of this test.




The *E. coli* standards are shown as circles with average values in the center and their upper and lower 95% confidence limits. Sample values from the autosampler bottles are shown as columns. As you can see, the confidence limits are quite large for high bacteria concentrations as shown in standard #3. This is because of high dilutions that require multiplication of results by the appropriate factors. IDEXX's Quantitray 2000 has tighter confidence limits than standard most probable number (MPN) tests and requires less dilution than membrane filtration.

CONCLUSION:
All samples through the autosampler fell within the 95% confidence limits of their corresponding standards. One concern before this test was whether or not a high bacteria sample would contaminate the sample tubing for subsequent samples. *As* seen in the chart above, sample #3 did not appear to affect samples #4 through #7.

It appears from this successful pilot test that autosamplers can be used for sampling storm events, provided that analysis can occur within 24 hours of the first sample time. Small concentrations of bacteria may remain in the sample tubing, but this is insignificant for storm events with *E. coli* concentrations typically in the thousands to ten thousands range, plus potential cross-contamination of samples can be minimized by programming the autosampler to rinse the sample tube at least once prior to obtaining a sample.

## Appendix D

## COLLECTION DATA LOG SHEET

Site Name: Seabrook
Type of Sample: Stormwater

| Sample ID | Date (ddmmyy) | Time | Flow (L/S) | Notes |
|---|---|---|---|---|
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |

In= Influent Ef= Effluent BLANK=Field Blank

Sampled by:

Parameters: E. coli, Fecal Coliform, Enterococci, pH, conductivity

Comments:

Delivered to lab by:
Date:
Time:
Received by:

Start Date and Time Bacterial analysis:
Read Date and Time Bacterial analysis:

**SAMPLE**
**COLLECTION DATA LOG SHEET**

Site Name: Seabrook
Type of Sample: Stormwater

| Sample ID | Date (ddmmyy) | Time | Flow (L/S) | Notes |
|---|---|---|---|---|
| IN1 | 300503 | 12:21 | 523 | |
| IN2 | 300503 | 13:21 | 1002 | |
| INREP1 | 300503 | 14:00 | 1200 | |
| INREP2 | 300503 | 14:00 | 1200 | |
| IN3 | 300503 | 14:21 | 2100 | |
| IN4 | 300503 | 15:21 | 1255 | |
| IN5 | 300503 | 16:21 | 219 | |
| EF1 | 300503 | 12:26 | 523 | |
| EF2 | 300503 | 13:26 | 1002 | |
| EFREP1 | 300503 | 14:00 | 1050 | |
| EFREP2 | 300503 | 14:00 | 1050 | |
| EF3 | 300503 | 14:26 | 2100 | |
| EF4 | 300503 | 15:26 | 1255 | |
| EF5 | 300503 | 14:00 | 1003 | |
| BLANK | 300503 | | | |
| | | | | |
| | | | | |
| | | | | |

In= Influent Ef= Effluent BLANK= FIELD BLANK

Sampled by: Scott Nolan

Parameters: E. coli, Fecal Coliform, Enterococci, pH, conductivity
Comments:

Delivered to lab by: Scott Nolan
Date: 31 May 03
Time: 10:05
Received by: Scott Nolan

Date and Start time Bacterial analysis: 31 May 03 12:02
Read Date and Time Bacterial analysis: 1June 03 12:05

**Appendix E**

# Time Interval Log Sheet

| Event # | Date | Forecasted Duration of Storm (hrs) | # of Paired Influent/Effluent Sample | Time Interval (min) | Initials |
|---|---|---|---|---|---|
| 1 | | | | | |
| 2 | | | | | |
| 3 | | | | | |
| 4 | | | | | |
| 5 | | | | | |
| 6 | | | | | |
| 7 | | | | | |
| 8 | | | | | |
| 9 | | | | | |
| 10 | | | | | |
| 11 | | | | | |
| 12 | | | | | |
| 13 | | | | | |
| 14 | | | | | |
| 15 | | | | | |

**Appendix F**

# Quality Assurance Plan:

# Microbiology Laboratory at the UNH-Jackson Estuarine Laboratory

***September, 2002***

**Latest Revision**
June 18, 2003

Dr. Stephen H. Jones
**Jackson Estuarine Laboratory**
**University of New Hampshire**
**85 Adams Point Rd.**
**Durham, NH 03824**

JEL Microbiology Lab Director:
& QA Officer:

_____
Signature / Date
Stephen H. Jones

*November 26, 2002 version reviewed and approved by Arthur Clark, EPA, on 12/2/2002.*

**Microbiology Laboratory Quality Assurance Plan**
**Jackson Estuarine Laboratory**
November, 2002

The Jackson Estuarine Laboratory's Microbiology Laboratory is a research laboratory that supports a variety of different projects on an ongoing basis. The lab also does some analysis for contracts, but this is not the major activity of the lab. As part of these projects, the lab routinely analyzes environmental samples for a variety of different fecal indicator bacteria, including total and fecal coliforms, enterococci and *Escherichia coli*. The procedures for these analyses are described in an SOP recently updated in September, 2002 (see below: Jones and Bryant, 2002). Various types of environmental samples are processed for analysis, including sediments; soils, feces, wastewater and water, but the vast majority of samples processed are surface water. Other bacteria have also been of interest for some past projects, including various pathogenic vibrio species, *Clostridium perfringens*, and a variety of environmentally relevant pure and mixed cultures.

## *1. Laboratory organization and responsibility*

**Table 1. Personnel Responsibilities and Qualifications Relative to Microbiology Laboratory**

| Name and Title | Responsibilities | Immediate Supervisor |
|---|---|---|
| Steve Jones, Ph.D. Principal Investigator on all lab projects | Administration and oversight on all projects, personnel training, QA Manager on many projects | NA |
| Andrew Beach, Laboratory Technician | Collection of water samples for microbial analysis and data compilation on one project | Steve Jones |
| Tamara Bryant, Research Technician II | QA Development Officer for ribotyping projects; training of student workers | Steve Jones |
| Danielle Morin, Laboratory Technician | Collection of water samples for microbial analysis and data compilation on one project | Steve Jones |
| Scott Nolan, Laboratory Technician | Collection of water samples for microbial analysis and data compilation on one project | Steve Jones |
| Bethany O'Hara, Research Technician II | Ribotyping technician and QA of laboratory equipment. | Steve Jones |
| Acksone Soumpholphakdy, Laboratory Technician | Collection of water samples for microbial analysis and data compilation on numerous projects. Training new students. | Steve Jones |

Based on EPA-NE Worksheet #6.

Dr. Jones is the QA manager for most projects and is responsible for ensuring the production of valid measurements and the routine assessment of measurement systems for precision and accuracy (e.g., internal audits and reviews of the implementation of the QA plan and its requirements). The development of QA procedures for ribotyping is an effort headed by Tamara Bryant, with supervision by Dr. Jones.

All job descriptions and employee qualifications are on file in Dr. Jones' office. All personnel are trained by those identified above for different projects to keep personnel updated on regulations and methodology. Dr. Jones keeps records on all the training that personnel receive outside of the laboratory.

***List of SOPs with the dates of the most recent revisions***

**Stephen H. Jones & Tamara Bryant.** Standard Procedure for Detection of Total Coliforms, Fecal coliforms, *Escherichia coli* and Enterococci from Environmental Samples. Revised: September, 2002. (based on: APHA, 1998; US EPA, 1986; 1996).

**Stephen H. Jones**. 1992. Most probable number method for the enumeration of *Clostridium perfringens* in marine sediments, p. 384-387. In, Standard Operating Procedures and Field Methods Used for Conducting Ecological Risk Assessment Case Studies, Mueller, C. et al. (Eds.). Naval Construction Battalion Center, Davisville, RI and Naval Shipyard, Portsmouth, NH. (based on: US EPA, 1996; Calise, 1991; St. John et al., 1982).

Copies of the SOP are on file in Dr. Jones' office and in the main laboratory. All listed SOPs are all reviewed annually and/or revised as changes are made.

### *3. Field sampling procedures*

Microbiological sampling from the field requires sterile containers, either autoclaved plastic bottles with caps or WhirlPak bags. The plastic bottles can be reused, so cleaning involves re-autoclaving for disinfection, thorough cleaning with soap and hot water then rinsing in tap water and deionized water. Surface sediment samples are collected using sterile scoops to remove surface sediment samples that are transferred to WhirlPak bags.

In general, the time interval between water sample collection and analysis is minimized to optimize the reliability of the analytical results. All samples are temporarily stored on ice in coolers in the field to reduce biological activity and changes in the microflora. Water samples can only be held for a total of 8 hours prior to analysis, or, 6 h maximum for transport to the laboratory and 2 h maximum time between arrival of sample at lab and analysis (APHA, 1998). For some projects where screening of samples is done to see generally what levels of bacteria exist, samples may be held for somewhat longer time intervals. All samples are stored in a refrigerator for at until the next day following the initial analysis to allow for re-analysis if the initial analysis was not acceptable for any reason. Data from reanalyzed sample results are flagged and only used for informational purposes. The only time custody forms are required is for projects other than internal projects, where another collaborative entity may require such forms.

All sample containers are checked just prior to analysis to ensure proper labeling, proper containment and that no cross contamination has occurred.

### *4. Laboratory sample handling procedures*

Bound laboratory notebooks are used for entering sample information into the laboratory records. Information is filled out in ink, dated and the person entering the information includes their name on the page(s). These notebooks are stored in the analytical laboratory and records throughout the holding time of the samples are maintained in them. After each batch of samples has been analyzed, the results are recorded into spreadsheet databases on a computer in a room adjacent to the analytical laboratory.

All unprocessed and processed samples are stored in designated areas within a walk-in cooler located adjacent to the analytical area of the laboratory. The temperature of the walk-in cooler is thermostatically controlled to be 4°C but actually ranges between 3-8°C; a chart recorder maintains a record of actual temperatures. UNH facilities personnel periodically check the cooler and maintain it. Unprocessed and processed samples are stored separately in the cooler, with unprocessed samples remaining in field coolers on the floor and processed samples stored on shelves. All sampling occurs according to predetermined schedules to ensure that holding times will not be exceeded and that incubations and final analyses will occur according to SOP requirements.

Chain-of-Custody procedures are not normally imposed because samples likely to be the basis for an enforcement action are not analyzed in this laboratory. However, occasional samples are received for analysis from other entities that may require Chain of Custody procedures for their own purposes.

Samples collected by other entities and delivered to the JEL Microbiology lab may be rejected if it is determined that they do not meet shipping, holding time and/or preservation requirements. This is determined by review of the datasheet provided to them by our laboratory to see when samples were collected and how they were shipped. Sample originators are immediately notified either by telling the delivery person or emailing/telephoning and providing them with the reasons for the rejection.

### *5. Calibration procedures for chemistry*

There are no chemical analyses performed by the Microbiology Laboratory.

### *6. Data reduction, validation, reporting and verification*

Data in laboratory notebooks are reviewed to ensure completeness of data entry and accuracy of labeling as soon as final analytical results are made. Within a few days, the raw data in the laboratory notebook are initially subject to calculation of average values from laboratory duplicate and any field duplicate analytical results. Two technicians working together conduct this calculation process. The sample average is recorded directly into the laboratory notebook. Sample averages are entered into spreadsheet databases for each project by two technicians: one reads the values from the lab notebook and relates the values to the other who enters the data into the computer. The project database(s) is organized by bacterial indicator, date and sample site, along with any other pertinent sampling date and site-specific data, measured or observed.

Dr. Jones is responsible for evaluating all data. This process includes assessment of database completeness, transcription errors and compliance with procedures. When possible, the data are also evaluated for consistency with previous correlated databases to determine if data are within expected ranges for sites and time of year. Omissions of data in spreadsheets will trigger a search of raw datasheets for missing data or possibly reanalysis of the questionable sample, if possible. If reanalysis is not possible or if data remain missing, invalid or otherwise affected entries will not be incorporated into the useable data set. When results appear to be abnormal, all appropriate project participants will review the available data and discuss the problem in periodic meetings to attempt to identify potential problems in sampling or analyses.

The reporting of analytical results is project dependent. For internal research projects, the data are fully analyzed by the PI and appropriate project technicians or graduate students, and eventually published in reports provided to the funding agency. For contract analysis results, the data are provided to funding agencies in Excel spreadsheets in formats pre-determined by the agency or project participants.

Each quantification procedure for the different bacterial indicators has specific verification procedures that are followed, and the procedures used at JEL are exactly as described in APHA (1998) . Counts are then adjusted based on the percent verification of these results.

Membrane filtration: In general, membrane filtration method verification procedures all require monthly verification of the identity of 10 colonies from one positive sample, as well as representative colonies of non-positive reactions or morphologies. All positive and negative total coliform, fecal coliform and *E. coli* colonies are verified by inoculation of LT and EC-MUG broths to check for lactose fermentation at 35°C, lactose fermentation at 44.5°C and b-glucuronidase activity. For enterococci verification, the colonies are streaked to BHI agar, growth is transferred to BHI broth. The24 h suspension is tested for catalase activity using H2O2 and checked microscopically for cocci and gram

stain. Catalse negative, gram positive cocci cultures are then transferred to bile esculin agar (35°C), BHI broth (44.5°C) and BHI broth + 6.5fiNaCl (35°C) to verify cultures as fecal streptococci and enterococci.

Multiple tube fermentation: In general, all MTF procedures are verified by using 10% of positive samples. TC, FC and Ec tests are verified using brilliant green and EC-MUG broths as described in SM 9221 B.3. *C. perfringens* tests are verified by streaking positive tubes to mCP agar and confirming *C. perfringens* by observing characteristic colonies after 24 h of anaerobic incubation at 44.5°C.

## *7. Quality control*

a. Within Sample Batches

Positive and negative samples are to be run with each sample set. These include positive samples of enterococci, total coliforms, fecal coliform and *E. coli,* either *Enterococcus faecalis* or *E. coli*. Negative sample cultures for the fecal indicator bacteria or other target bacteria (vibrio species, *Clostridium perfringens*, etc.) are selected from a variety of different non-fecal and non-target bacterial species that are maintained in the laboratory. In each sample set, duplicate analyses of a positive sample are run by the analyst. Colony counts are expected to agree within 5%. Monthly positive samples are also run in duplicate by the different analysts, and colony counts between analysts are expected to agree within 10%.

b. Precision

Precision for bacterial indicator measurements is typically determined according to Standard Methods 9020 B-8. (APHA, 1998). The range (R) for duplicate samples is calculated and compared to predetermined precision criteria. The precision criterion is calculated from the range of log-transformed results for 15 duplicate according to the following formula:

3.27 × (mean of log ranges for 15 duplicates) = precision criterion

The precision criterion is updated periodically using the first 15 duplicate samples analyzed in a month by the same analyst. If the range of ensuing pairs of duplicate samples is greater than the precision criterion, then the increase in imprecision will be evaluated to determine if it is acceptable. If not, analytical results obtained since the previous precision check will be evaluated and potentially discarded. The cause of the imprecision will be identified and resolved.

c. Media Preparation and Equipment

Various types of sterility controls are included in the different procedures used to detect and enumerate microorganisms. Sterile water is filtered through membrane filters in filter towers prior to use of the filter tower for sample filtration for the first and last samples of a sample batch. The membrane filter is then incubated on the target test media to see if any bacteria are present. Uninoculated dilution tubes and agar media are incubated along with inoculated media to check for contamination for each batch of samples. If the results of the positive or negative controls indicate either contamination or culture problems, all sample results will be discarded and samples will be reanalyzed, if holding time requirements are not exceeded.

Other QC procedures for lab supplies generally follow SM 9020 B.4 for pH and inhibitory substances on glassware, laboratory reagent water quality, quality of media and reagents and membrane filter integrity. Procedures for preparing, sterilizing, handling and storing media and other equipment are as described in SM 9020 B.4i.1-5.

## *8. Schedule of internal audits*

Dr. Jones conducts periodic (minimum frequency: annually for projects >1 year in duration) internal audits of all aspects of project QA/QC and personnel performance. The timing of performance audits is project specific, and typically occurs in the very beginning of a project, within one month of project analysis initiation, and later in the project after the technicians have established procedural prowess. Any problems are noted, corrective actions are recommended and follow-up audits are conducted to verify compliance with correct procedures. Written records in the form of checklists with details of problems and follow-up audit results are kept in Dr. Jones' office.

### *9. Preventive maintenance procedures and schedules*

The technicians responsible for project or laboratory QC conduct all maintenance and inspection of equipment based on manufacture requirements and specifications. Every day a piece of equipment is used it receives a general inspection for obvious problems. The most common assessment requiring corrective action is maintenance of correct temperatures for incubators. Results of inspections are recorded on datasheets that include date, time, and inspector initials, and completed sheets are on file in Dr. Jones' office. Much of the other equipment used in the Microbiology Lab is not under the direct control of Dr. Jones and is maintained by regular UNH inspections (Autoclave, walk-in coolers, scales, etc.). Lab technicians always check chart recorders and digital read outs on the autoclave and the coolers with each use to confirm correct settings and conditions. Any probelms are reported to the JEL Lab Manager who contact UNH Maintenance for any necessary repairs beyond his expertise. Scales are checked annually by UNH-hired experts and the date, time, results and inspector's initials are recorded on the scale. In addition, microbiological data are inspected within a few days of sample analysis to allow instrument (or user) malfunctions to be caught quickly, and corrected as needed.

### *10. Corrective action contingencies and record keeping procedures*

Unacceptable lab QC checks triggers immediate review of analytical procedures, sample processing and equipment with the technicians involved. Data results from the time period between the previous acceptable lab QC checks are reviewed to determine if there is evidence for accepting the data, otherwise, it is considered invalid. All project-specific personnel are responsible for participating in corrective actions like re-training or learning modified QC procedures to ensure future acceptability. A database of corrective actions is maintained on a computer in the PI's office. The office is either occupied by the PI or is locked and no one else is admitted in.

REFERENCES

American Public Health Association. (APHA). 1998. Standard Methods for the Examination of Water and Wastewater. 20th Edition. American Public Health Association, Washington, DC.

Calise, B.A. 1991. ERL-N Standard Operating procedure. Most probably number method for enumeration of *Clostridium perfringens* in marine waters. ERL-N SOP 1.03.017, 4 pp.

St. John, W.D., J.R. Matches and M.M. Wekell. 1982. Use of iron milk medium for enumeration of *Clostridium perfringens*. J. Assoc. Anal. Chem. 65: 1129-1133.

U.S. Environmental Protection Agency (USEPA). 1996. ICR Microbial Laboratory Manual. Sections X (*E. coli*) and XI (*C. perfringens*). EPA 600/R-95/178. Environmental Protection Agency, Office of Research and Development, Washington, DC.

U.S. Environmental Protection Agency (USEPA). 1986. Test methods for *Escherichia coli* and enterococci by the membrane filtration procedure. EPA 600/4-85/076. Environmental Protection Agency, Environmental Monitoring and Support Laboratory, Cincinnati, OH.